

U.S.S.N. 09/544,045

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AMENDMENT AND RESPONSE TO OFFICE ACTION

In the Claims

1. (original) A method of identifying variant recombinases that mediate recombination at variant recombination sites, the method comprising,

(a) bringing into contact

a mutant recombinase,

a first nucleic acid sequence comprising a first reporter gene and first and second recombination sites, wherein the first and second recombination sites are variant recombination sites, and

a second nucleic acid sequence comprising a second reporter gene and third and fourth recombination sites, wherein the third and fourth recombination sites can be recombined by a non-mutant recombinase,

(b) determining if recombination occurs between the first and second recombination sites, and determining if recombination occurs between the third and fourth recombination sites,

wherein recombination between the first and second recombination sites indicates that the mutant recombinase is a variant recombinase that mediates recombination at variant recombination sites,

wherein recombination between the third and fourth recombination sites indicates that the mutant recombinase retains the ability to mediate recombination at non-variant recombination sites.

2. (previously amended) The method of claim 1 wherein the recombination sites comprise recognition sequences and compatibility sequences,

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wherein the recognition sequences of the first and second recombination sites differ from the recognition sequences of the third and fourth recombination sites,

wherein the compatibility sequences of the first and second recombination sites are sufficiently similar to allow recombination between the first and second recombination sites, and wherein the compatibility sequences of the third and fourth recombination sites are sufficiently similar to allow recombination between the third and fourth recombination sites, and

wherein the compatibility sequences of the first and second recombination sites differ from the compatibility sequences of the third and fourth recombination sites such that neither the first nor the second recombination site can be recombined with either the third or the fourth recombination site.

3. (previously amended) The method of claim 1 wherein recombination frequency between the first and second recombination sites mediated by a non-mutant recombinase is significantly reduced.

4. (original) The method of claim 1 or 2 wherein the first and second recombination sites have identical sequences, and wherein the third and fourth recombination sites have identical sequences.

5. (original) The method of claim 1 wherein recombination between the first and second recombination sites alters the expression of the first reporter gene, wherein recombination between the first and second recombination sites is determined by determining if expression of the first reporter gene is altered, and

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wherein recombination between the third and fourth recombination sites alters the expression of the second reporter gene, wherein recombination between the third and fourth recombination sites is determined by determining if expression of the second reporter gene is altered.

6. (original) The method of claim 5 wherein recombination between the first and second recombination sites allows the first reporter gene to be expressed.

7. (original) The method of claim 6 wherein the first nucleic acid sequence further comprises a spacer sequence flanked by the first and second recombination sites, wherein the spacer sequence interrupts the first reporter gene such that the first reporter gene is not expressed, wherein recombination of the first and second recombination sites excises the spacer sequence which allows the first reporter gene to be expressed.

8. (original) The method of claim 6 wherein a portion of the first reporter gene is inverted, wherein the inverted portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the inverted portion of the first reporter gene which allows the first reporter gene to be expressed.

9. (original) The method of claim 5 wherein recombination between the first and second recombination sites prevents expression of the first reporter gene.

10. (original) The method of claim 9 wherein the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites excises the first reporter gene which prevents expression of the first reporter gene.

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11. (original) The method of claim 9 wherein a portion of the first reporter gene is flanked by the first and second recombination sites, wherein recombination of the first and second recombination sites inverts the flanked portion of the first reporter gene which prevents expression of the first reporter gene.

12. (original) The method of claim 5 wherein recombination between the third and fourth recombination sites allows the second reporter gene to be expressed.

13. (original) The method of claim 12 wherein the second nucleic acid sequence further comprises a spacer sequence flanked by the third and fourth recombination sites, wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not expressed, wherein recombination of the third and fourth recombination sites excises the spacer sequence which allows the second reporter gene to be expressed.

14. (original) The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene is not transcribed.

15. (original) The method of claim 13 wherein the second reporter gene encodes a protein, wherein the spacer sequence interrupts the second reporter gene such that the protein encoded by the second reporter gene is not translated.

16. (original) The method of claim 13 wherein the spacer sequence interrupts the second reporter gene such that the second reporter gene produces an inactive expression product.

17. (original) The method of claim 12 wherein a portion of the second reporter gene is inverted, wherein the inverted portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites

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inverts the inverted portion of the second reporter gene which allows the second reporter gene to be expressed.

18. (original) The method of claim 5 wherein recombination between the third and fourth recombination sites prevents expression of the second reporter gene to be expressed.

19. (original) The method of claim 18 wherein the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites excises the second reporter gene which prevents expression of the second reporter gene.

20. (original) The method of claim 18 wherein a portion of the second reporter gene is flanked by the third and fourth recombination sites, wherein recombination of the third and fourth recombination sites inverts the flanked portion of the second reporter gene which prevents expression of the second reporter gene.

21. (original) The method of claim 1 wherein the first nucleic acid sequence is a first nucleic acid construct and the second nucleic acid sequence is on a second nucleic acid construct.

22. (original) The method of claim 21 wherein the first nucleic acid construct is an extrachromosomal vector and the second nucleic acid construct is in the genome of a host cell.

23. (original) The method of claim 1 wherein the first and second nucleic acid constructs are on the same nucleic acid construct.

24. (currently amended) A method for producing site-specific recombination of DNA, comprising,

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contacting a variant recombinase identified by the method of claim 1 with third and fourth DNA sequences,

wherein the third DNA sequence comprises a fifth recombination site and the fourth DNA sequence comprises a sixth recombination site,

wherein the variant recombinase mediates recombination between the fifth and sixth recombination sites thereby producing the site specific recombination,

wherein the fifth recombination site is selected from the group consisting of the first recombination site of claim 1, and the third recombination site of claim 1; and

wherein the sixth recombination site is selected from the group consisting of the second recombination site of claim 1, and the fourth recombination site of claim 1.

25. (previously amended) The method of claim 24 wherein the fifth recombination site, the sixth recombination site, or both, are variant recombination sites.

26. (previously amended) The method of claim 24, wherein the third and fourth DNA sequences are connected by a pre-selected DNA segment.

27. (previously amended) The method of claim 26, wherein the fifth and sixth recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

28. (original) The method of claim 27, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

29. (previously amended) The method of claim 27 further comprising contacting the variant recombinase with a fifth DNA sequence comprising a seventh recombination site,

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wherein the fourth and fifth DNA sequences are connected by a second pre-selected DNA segment.

30. (previously amended) The method of claim 29 wherein the fifth recombination site is a variant recombination site recognized by the variant recombinase and not by wild type recombinase, and wherein the sixth and seventh recombination sites are recombination sites recognized by wild type recombinase and by the variant recombinase.

31. (previously amended) The method of claim 30 further comprising, prior to contacting the variant recombinase with the fifth, sixth, and seventh recombination sites, contacting the recombination sites with wild type recombinase, thereby producing site specific recombination between the sixth and seventh recombination sites resulting in a deletion of the second pre-selected DNA segment.

32. (original) The method of claim 29, wherein the second pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

33. (previously amended) The method of claim 26, wherein the fifth and sixth recombination sites have opposite orientations and the site-specific recombination is an inversion of the nucleotide sequence of the pre-selected DNA segment.

34. (previously amended) The method of claim 33, wherein the fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

35. (original) The method of claim 33, wherein the pre-selected DNA segment is a gene for a structural protein, an enzyme, or a regulatory molecule.

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36. (previously amended) The method of claim 24, wherein the fourth and fifth DNA sequences are introduced into two different DNA molecules and the site-specific recombination is a reciprocal exchange of DNA segments proximate to the recombination sites.

37. (previously amended) The method of claim 36, wherein the fifth and sixth recombination sites are variant recombination sites recognized by the variant recombinase.

38. (previously amended) The method of claim 24 wherein the fourth DNA sequence includes a label, wherein recombination between the fifth and sixth recombination sites associates the label with the third DNA sequence.

39. (previously amended) The method of claim 38 wherein the third DNA sequence is a large circular DNA molecule.

40. (original) The method of claim 24 wherein recombination occurs in a cell.

41. (previously amended) The method of claim 40 wherein the variant recombinase is contacted with the third and fourth DNA sequences by introducing into the cell a sixth DNA sequence comprising DNA encoding the variant recombinase.

42. (previously amended) The method of claim 41, wherein the sixth DNA sequence further comprises a regulatory nucleotide sequence and expression of the variant recombinase is produced by activating the regulatory nucleotide sequence.

43. (original) The method of claim 40, wherein the cell is a eukaryotic cell, a mammalian cell, a yeast cell, a fungal cell, a prokaryotic cell, a bacterial cell, an archae bacterial cell, or a cell in a multicellular organism.

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44. (original) The method of claim 43, wherein the multicellular organism is a plant, an animal, or a mammal.

45. (previously amended) The method of claim 40, wherein the third and fourth DNA sequences are connected by a pre-selected DNA segment, wherein the first and second recombination sites have the same orientation and the site-specific recombination of DNA is a deletion of the pre-selected DNA segment.

46. (original) The method of claim 45, wherein the cell is a multicellular organism.

47. (original) The method of claim 45, wherein the pre-selected segment is an undesired marker or trait gene.

48. (original) The method of claim 24, wherein the variant recombinase is contacted with the recombination sites *in vitro*.

49. (original) The method of claim 48, wherein the method further comprises introducing the recombined DNA into a cell.